Fig. 1

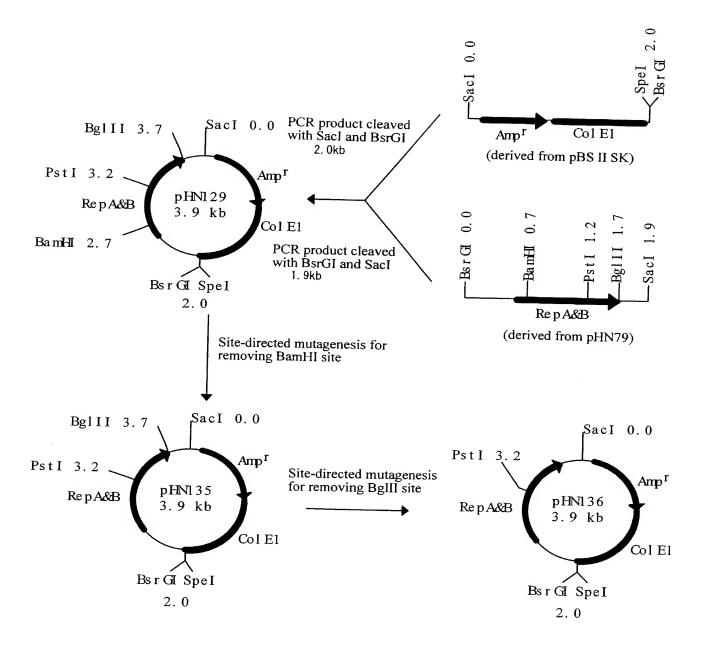
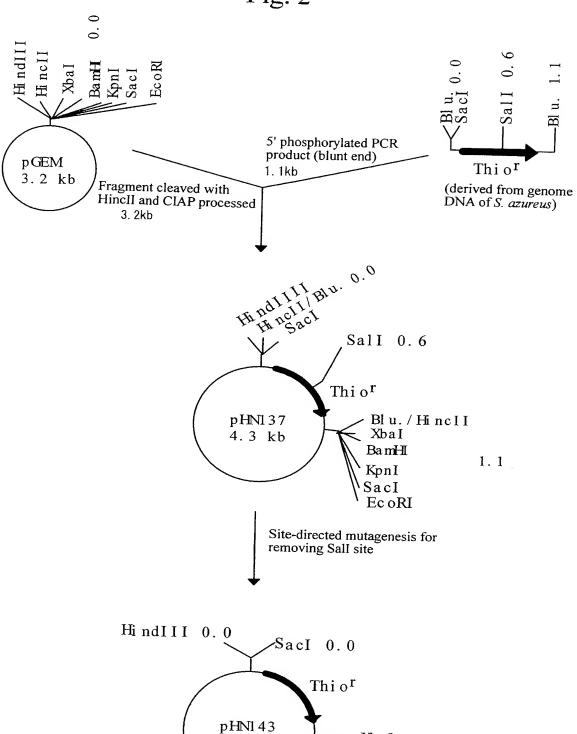


Fig. 2



4.3 kb

XbaI

BamHI

KpnI SacI EcoRI 1.1

Fig. 3

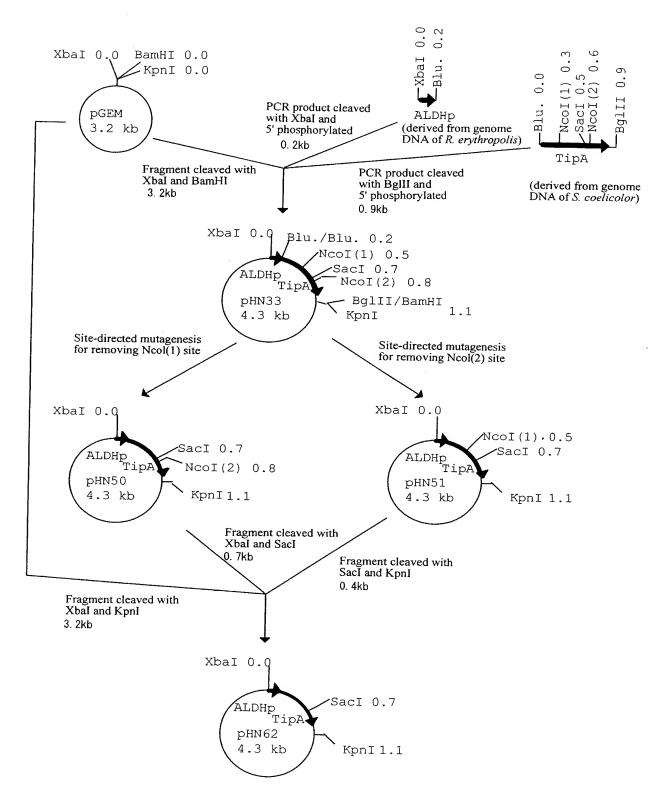


Fig. 4

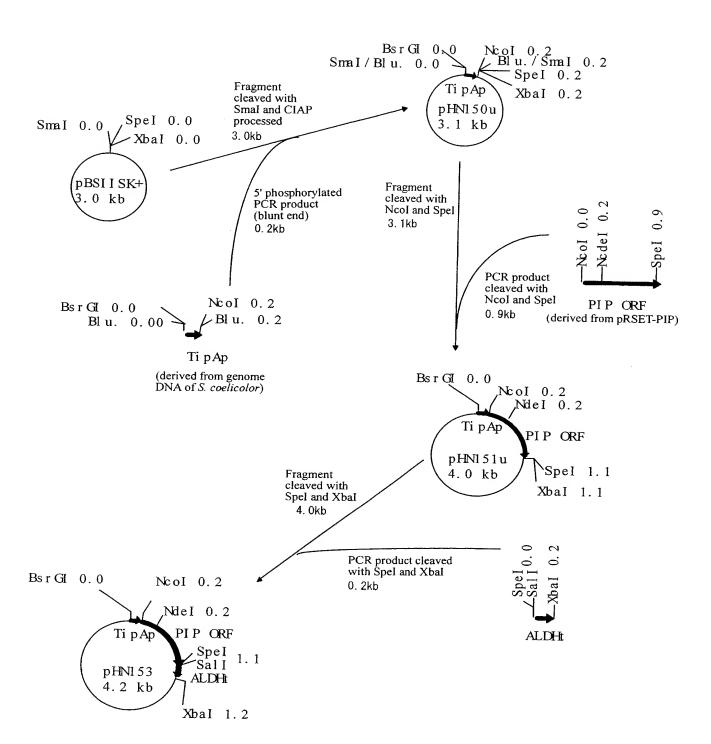
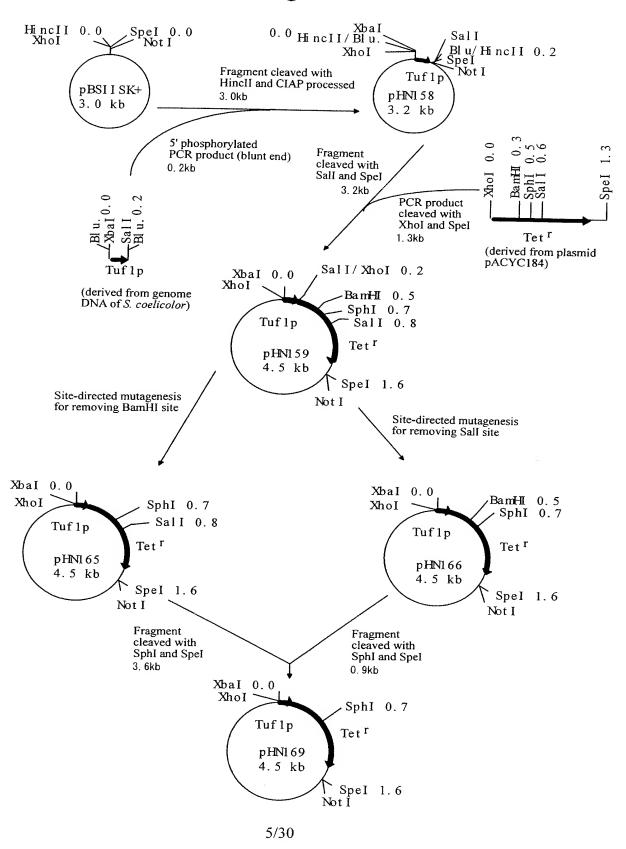
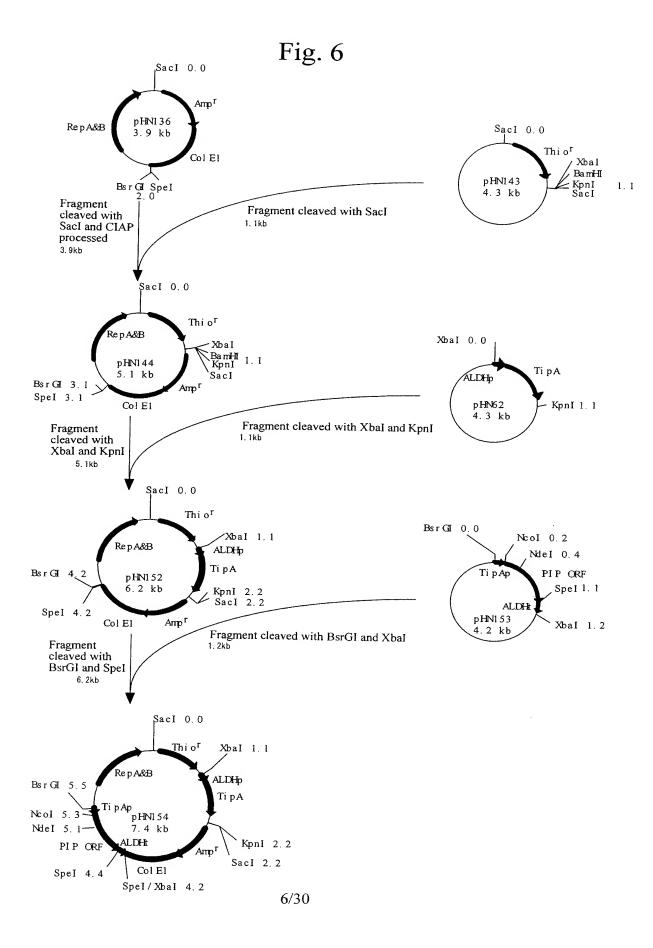


Fig. 5





Xbal 1.1 Tet ^r ALDH Ti pA KpnI 3.8 SacI 3.8 Sacl 0.0 Thi o^r pHNI 71 9.0 kb Amp Ti pA- LGI 0p RepA&B Col El Ndel 6.6 PIP ORF Ncol 6.8 Bsr Gl 7.0 SpeI 6. 0 PCR product cleaved with BsrGl and Ncol (derived from plasmid pHN170) Fragment cleaved with BsrGl and Ncol 8.8kb NcoI 0.2 Spel 1.6 Ti pA-LG10p Tet ^r BsrG 0.0 pHN169 4.5 kb Tuf 1p XbaI 0.0 - Xbal/Spel 2.7 Fragment cleaved with XbaI and Spel 1.6kb XbaI 1.1 KpnI 2.2 Sacl 2.2 Tet ^r ALDH XbaI 1.1 / Kpnl 3.8 Sacl 3.8 ALDHp Ti pA Thi or Amp Sacl 0.0 SacI 0.0 Thi or Spel/Xbal 4.2 pHNI 70 9. 0 kb Amp Ti pAp pHNI 54 7.4 kb Rep A&B Col El Col E1 RepA&B ALD:N ALDH Ti pAp Fragment cleaved with Xbal and CIAP processed Spel 4.4 PIP ORF PIP ORF Bsr G 5.5 Reol 5.31 Ndel 5.1-NcoI 6.8 NdeI 6.6 -Bsr Gf 7.0 Spel 6.0

Fig. 6 (continued)

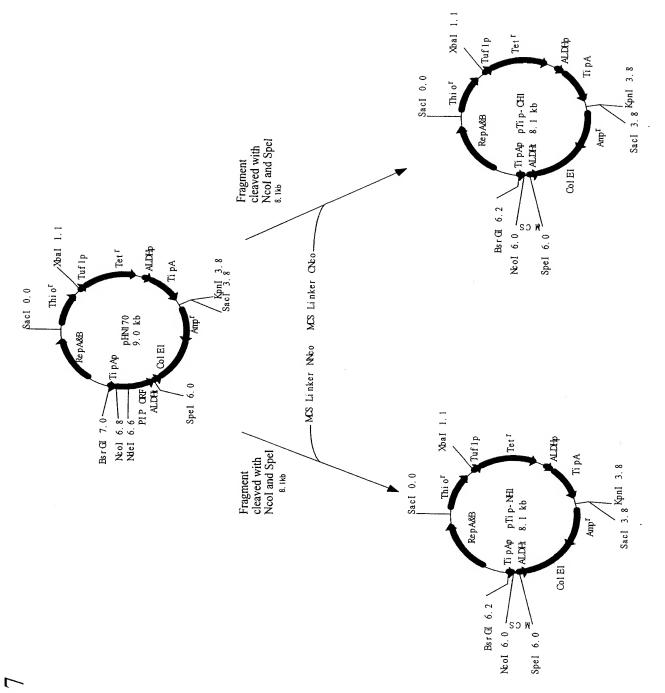


Fig.

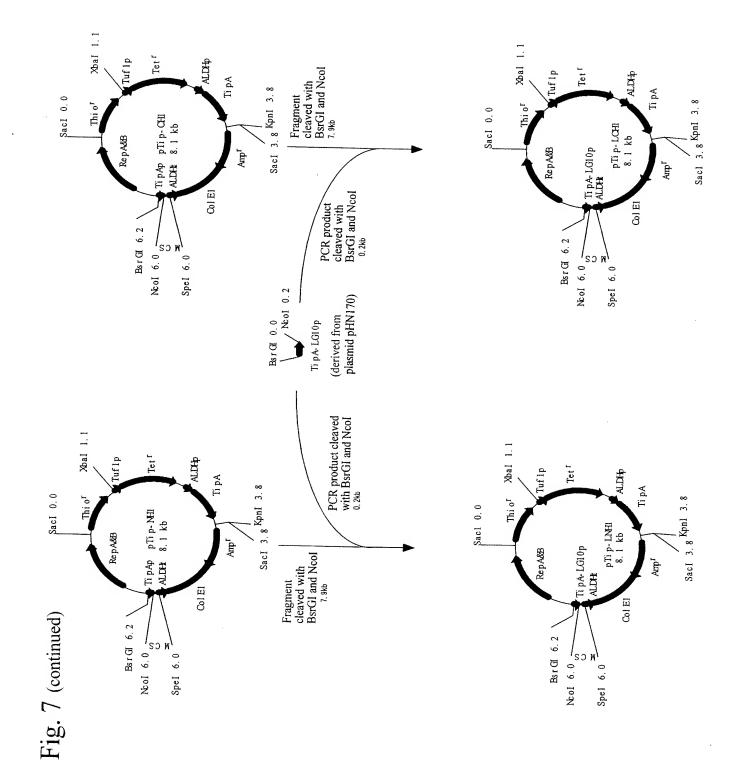
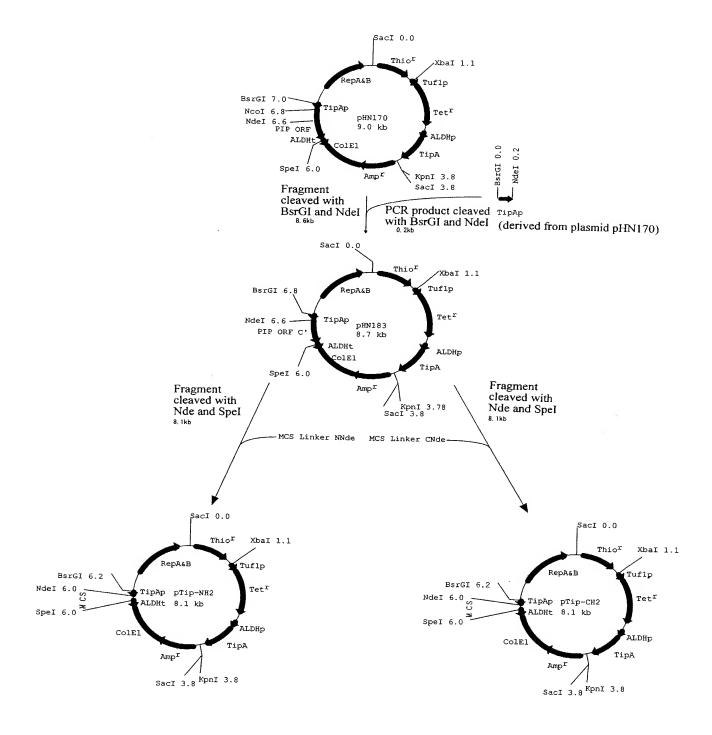


Fig. 8



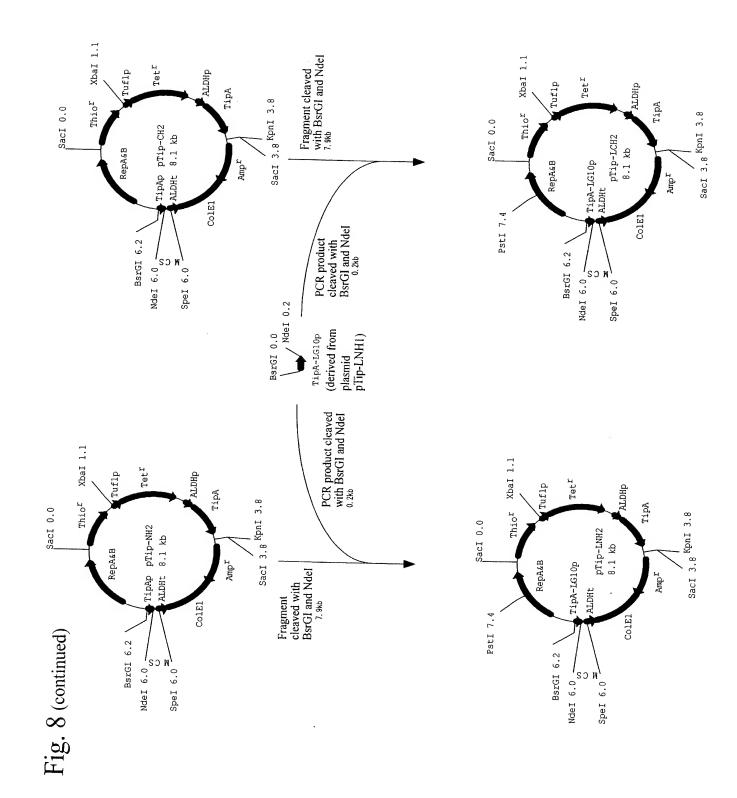
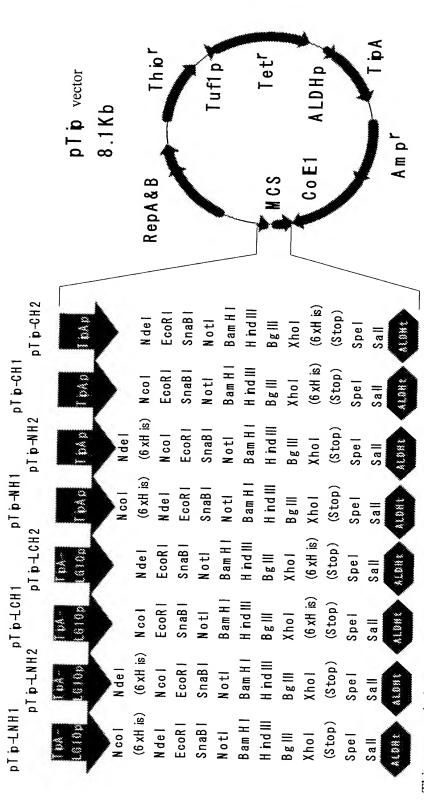


Fig. 9a



Thiostrepton induction system

Thio ^r= confers thiostrepton resistance to *R.erythropolis*

ALD Hp = promoter which constitutively produces TipA protein

That = encodes a TipA protein
That = TipA promoter

TipA 4.610p = improved TipA promoter ALDHt = transcription termination sequence

Regions necessary for the autonomous replication of a plasmid co E1 = for E. coli

RepA&B = for R. erythropolis

Antibiotic resistance marker

Tuflp-Tet' = transformation marker for R.erythropolis

Am p^r = transformation marker for E. coli

 $\frac{Bs r G1}{G1G}$ GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC GCC CGG GCT GAG GGA GCC GAC

GGC ACG CGG CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC ITTG CAG CTC -35

ACG TCA CGT GAG GAG GCG TGG ACG GQG TCA GAG AAG GGA GCG GCQ ATG RBS

GTC TAG AAA TAA TIT TGT TTA ACT TTA AGA AGG AGA TAT ACC

GGC CAC CAT ATG GGA ATT CTA CGT AGC GGC CGC GGA TCC GIV His His His His Met GIV IIe Leu Arg Ser GIV Arg GIV Ser

AAG CTT AGA TCT CGA GGA TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG CCC

CTC GCT GCG GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA

CAC ACG GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC GTT

CCA CCC CGG TTG GTG ACC ACC GCT GGG GCT GAC CTG CTA CGC CGC CTC

AGC GGG ACT CTA GT

 $\frac{Bsr61}{6$ TG TAC ATA TCG AGG CGG GCT CCC ACG GCC CGG GCT GAG GGA GCC GAC

GGC ACG CGG CGC CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC TTG CAG CTC

ACG TCA CGT GAG GAG GCG TGG ACG GQG TCA GAG AAG GGA GCG GCQ ATG RBS

GTC TAG AAA TAA TTT TGT TTA ACT TTA AGA AGG AGA TAT ACC

GGA ATT CTA CGT AGC GGC CGC GGA TCC AAG CTT AGA TCT CGA GGA CAT CAC GIY lie Leu Arg Ser Gly Arg Gly Ser Lys Leu Arg Ser Arg Gly His His

CAT CAC CAT CAC TGA ACT AGT CGA CCC ACC GGC ACC CGT GAG CCC CTC GCT

GCG GGT GCC GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA CAC ACG

GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC GTT CCA CCC

CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC CTC AGC GGG

ACT CTA GT

BS/GI GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC GGC CGG GCT GAG GGA GCC GAC GGC ACG CGG CGG CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC TTG CAC CTC

ACG TCA CGT GAG GAG GCG TGG ACG GCG TCA GAG AAG GGA GCG CAT ATG RBS

G TCT AGA AAT AAT TIT GIT TAA CTT TAA GAA GGA GAT ATA CAT

GGC CAT CAC CAT CAC CAT CAC GCC ATG GGA ATT CTA CGT AGC GGC CGC GGA GIY His His His His Ala Met GIY IIe Leu Arg Ser GIY Arg GIY

CCC CTC GCT GCG GGT GCG GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC

AAA CAC AGG GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC

GTT CCA CCC CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC

CTC AGC GGG ACT CTA GT

BS/GI GTG TAC ATA TCG AGG CGG GCT CCC ACG GCC CGG GCT GAG GGA GCC GAC GGC ACG CGG CTC ACG GCG TGG CAC GCG GAA CGT CCG GGC ITG CACI CTC

ACG TCA CGT GAG GAG GCG TBG ACG GCG TCA GAG AAG GGA GCG CAT ATG TCT TAA GAA GAA GAT ATA TTT GTT TAA CTT TAA GAA GGA GAT ATA CAT HOUSE CAR SAAR AAT AAT TTT GTT TAA GAA GAA GAA ATA CAT AATA CAT AATA CAT AATA CAT AAATA CATA CATA

GGA ATT CTA CGT AGC CGC CGC GGA TCC AAG CTT AGA TCT CGA GGA CAT CAC GIY lie Leu Arg Ser Gly Arg Gly Ser Lys Leu Arg Ser Arg Gly His His

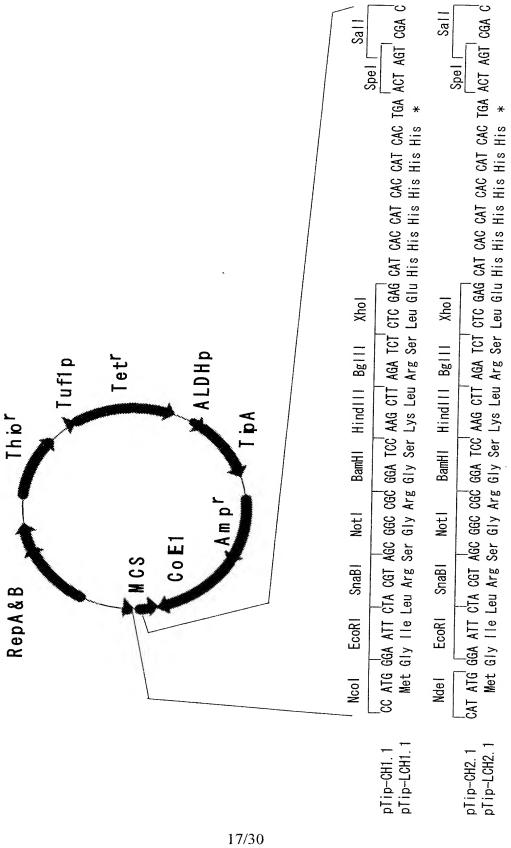
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GOG GGT GCC GGT GCG AGG GAC TGC AAC ACG CGA AAC CTG CAC AAA CAC ACG

GAG GTT GGA ATG AGC GCC ACG GAC ACA CCC GAT ACC GGC GCC GTT CCA CCC

CGG TTG GTG ACC ACC GCT GGG GCG GCT GAC CTG CTA CGC CGC CTC AGC GGG

Fig. 10



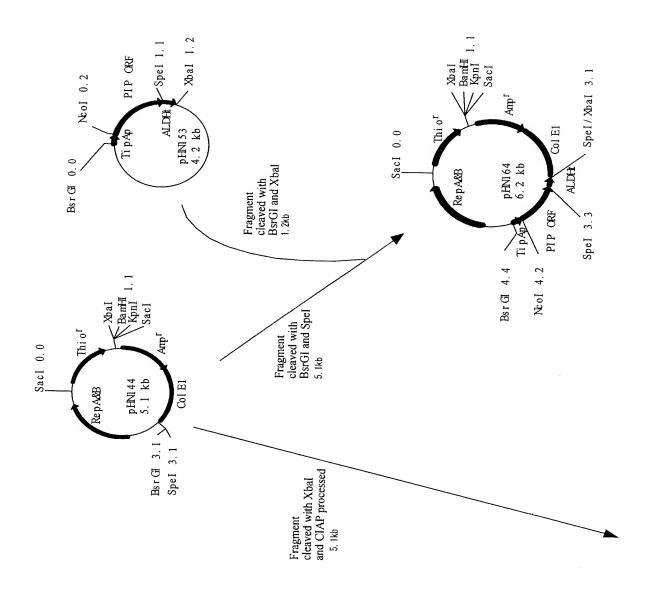
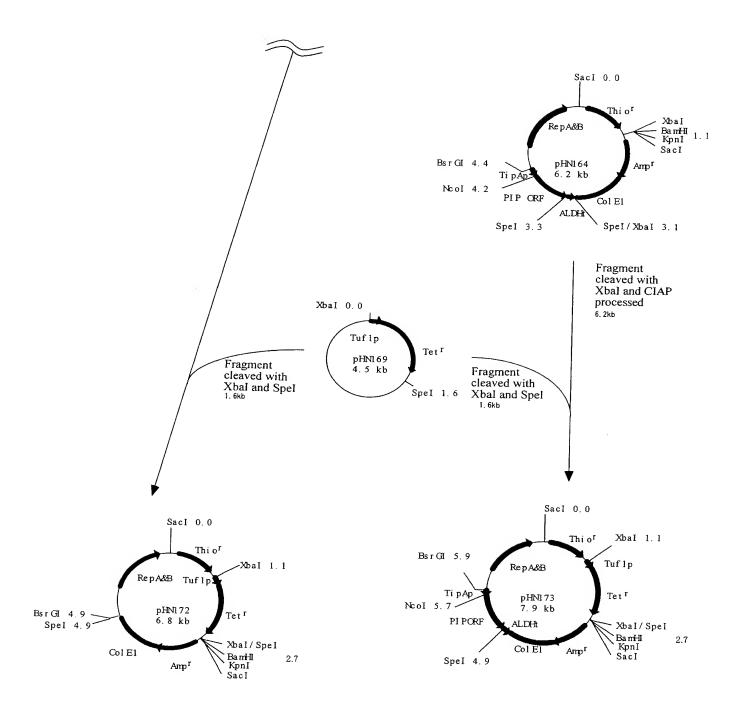
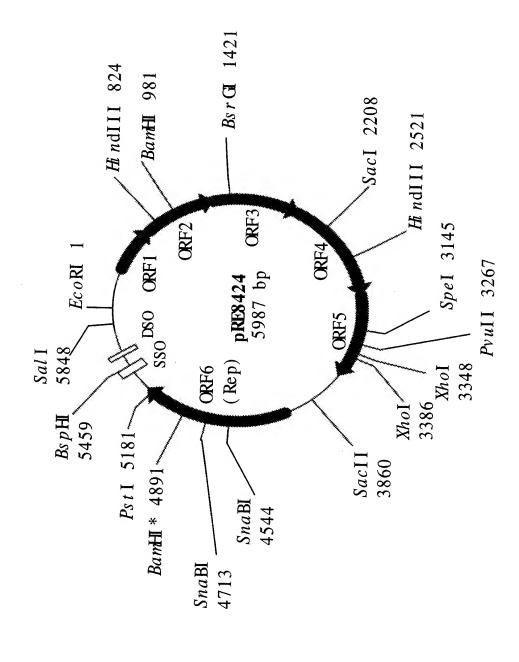


Fig. 11 (continued)



cgcccgggctgagggagccgacggcggcggctcacggcgtggcacgcggaacgtccgggcttgcacctcacgtc acgtgaggaggcagcgtggacggcgtcagagaagggagc RBS ggccatg

Fig. 13



Motif III	l a XYXXKXq X	LAAMLTKI AS I GNYVSKAQT MATYLAKGAS LI BYLTKAQD LABYI AKTQD LABYI AKTQD
		53 67 54 80 69
Motif II	g XXg XXr a Xe Xt Xg XXn GwHXHXh Xl X	GCDGYVRAVEI THGK- NGWHVHVHALL GL VGYVRANEI THGK- HGWHVHSHVLI GL VGYVRANEI THGK- HGWHVHSHVLI VEHITYSDYEVIDS WA- NGWHLHRNM.L S4 MATIYLAKGNG GYI GWRAAEVIRSKKNGYHPHLNLLV S9 LAFYLTKNQD GYVGM RATEVIVOQI NGWHPHI HAI V 69 LAFYL AKTOD
		33 34 77 59
Motif I	Xvt XTXRH	 26 MYTMITMRH 33 27 MLTLTQRH 33 76 MFVGTVRH 34 27 LVIFTARH 77 27 LVIFTARH 59 27 LVIFTARH 59 27 LVIFTARH 59
Motif IV	GLXXCGXXWXCPXC	GLRSCCKGW CPCC GLHTCGSVWACPVC GLVRCCRI WFCPEC GLMRCCRI WLCPVC GLMRCCRI WLCPVC
		68 138 38 20 20
	Consensus	pRE8424 pAP1 pBL1 pJV1 pIJ101 pSN22

C-terminal motif

Wey EXa XXgr Rai XWr glr	276 WREFEFGSMGRRAI AWSRGLR	WEYEKASFGRRALTWSKGLR	WREYEVGSKNLRS-SWSRGAK	WAQYEEALAGRRAI EWIRGLR	WHEYERATRGRRAI EWIRYLR	WHEYERATKGRRAI EWIRYLR	· · · * · * * · · *
	276	365	250	352	288	288	
Consensus	pRE8424	pAP1	pBL1	pJ Vl	pIJ101	pSN22	

```
CGAKERGANGCE-KAN-CECTORCE---KAKETGGGGGAG-
                                 GAJAATKAAA-CTGA-AGARETICTaaggaakcggca--
                                                                 GAGGGAAAA--CGGA-ACAGGTTT--CGGAAAAGAA--
                                                                                 GACCIONAMACETOTCCOCOCOCOTT - - COCAMAGAAA- -
                                                 CTGGGAMMANAGGGA--(AGGGT)---(MGGTMAAGGGTT
                 CAGGIATGC-G-GA-AAACTITT--IAGGAACAA---
                              1314
3375
1346
7805
 5705
               2378
p RE8 424
                                              pJ V1
pI J 1 0 1
p SN2 2
             pAP1
                              pBL1
```

DSO

Nicking site

Fig. 17

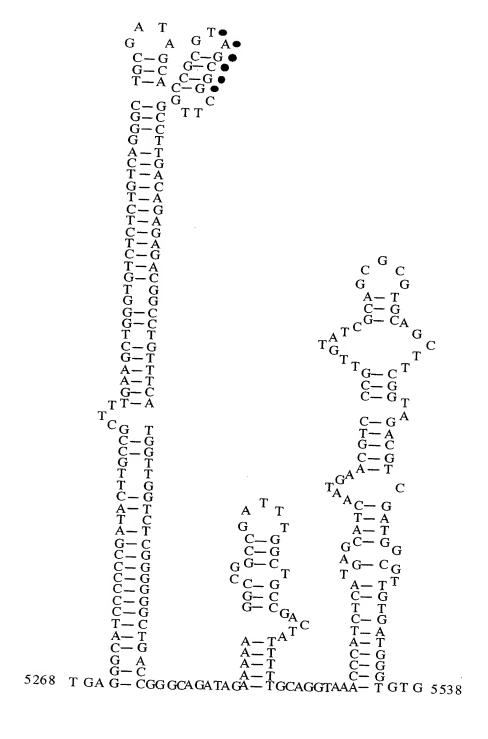


Fig. 18-1 Thio $Thio^{r}$ Sega Regist AgsA Augus 2998 X 2362 prip-qra prip-ora Prips. Pripa LG10RBS LG10RBS (8.2 %b) (8.2 kb) Tybes Typica ColE1 ColE1 $^{\circ}$ ThcA ThcAAugus. TipAL TipAL MCS type 1 MCS type 2 $Thio^{r}$ $Thio^{r}$ 2002 THEX grip-str prip-ari LG10RBS 🛨 LG10RBS (8.3 MM) (8,3 kb) T_{TRCA} Tanca ColE1 ColE1 ThcAThcAAmpa TipAL MCS type 2 MCS type 1 Thior $Thio^{r}$ Eggs. Segne Regard Alegoia CHI F Chiz. prip-oca prip-ocz PILIPA Friga LG10RBS LG10RBS (8.4 kb) (8.4 %8) Tanca Tabaa ColE1 TipAL TipAL MCS type 2 MCS type 1 $Thio^{r}$ Thior Chi z Chil F prip-acz Fright prip-RC1 MAKER LG10RBS (8.9 kb) LG10RBS (8.5 80) Tribers Tanca ColEi $^{P}ThcA$ ThcA Ampa Ango^x TipAL

26/30

MCS type 1

MCS type 2

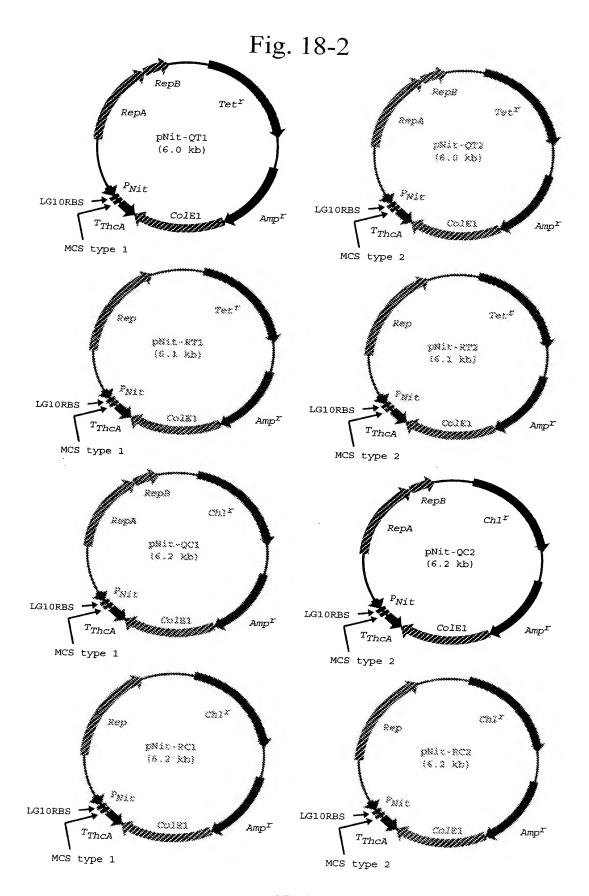


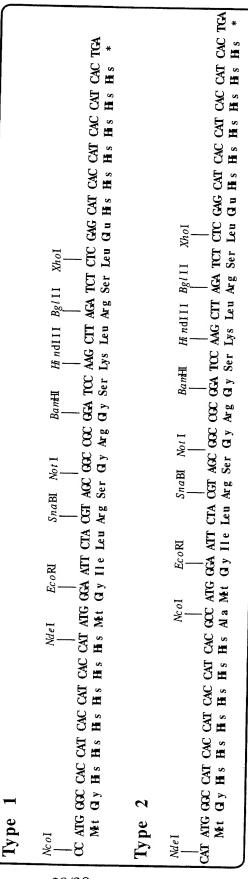
Fig. 19

Ti pA-LG10p or Nit-LG10p

: : : : THE TAC ATA TOG AGG COS GCT COCC ACG COCC COCC COS GCT GAG GCA COCC GAC GCC ACG COS COS CTC ACG COCC TGG CAC GCG GAA CGT COG GAC

TIG CAC CIC AGG TCA GGT GAG GAG GAG GGG AGG GGG TCT AGA AAT AAT TIT GIT TAA CIT TAA GAA GAA GAT ATA TA TAA T

MCS



ALDHt

Spel Sall

ÀCT AGT CCA CCC ACC GGC ACC CGT GAG CCC CTC GCT GGG GGT GCC GGT GGG GAC TGC AAC ACG CGA AAC CTG CAC AAA CAC AGG GAG GTT GGA ATG AGC GCC ACG GAC ACA GCC GAT ACC GCC GTT GCA CCC GTTG GTG ACC ACC GCT GCG GCT GAC CTG CTA CGC GCC CTC AGC GGG ACT CTA GT

Fig. 20

